Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/GB04/005443

International filing date: 22 December 2004 (22.12.2004)

Document type: Certified copy of priority document

Document details: Country/Office: GB
Number: 0329907.0
Filing date: 23 December 2003 (23.12.2003)

Date of receipt at the International Bureau: 08 February 2005 (08.02.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

PCT/GB2004/005443

INVESTOR IN PEOPLE

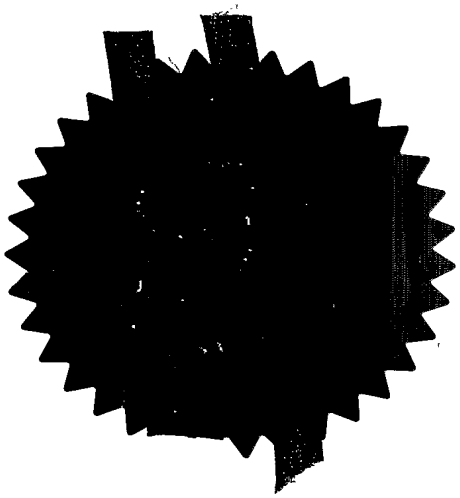
The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.



Signed Andrew Wright

Dated 19 January 2005

Patents Form 1/77

Patents Act 1977
(Rule 16)

The Patent Office

28DEC03 E861885-10 D00056
P01/7700 0.00-0329907.0 CHEQUE

THE PATENT OFFICE
C
23 DEC 2003
LONDON

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
South Wales
NP9 1RH

1. Your reference P36027GB/PWC

2. Patent application number
(The Patent Office will fill in this part) 0329907.0 23 DEC 2003

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Innomed Ltd
Craigmore, Douglas Road
Longniddry, East Lothian EH32 0LE
United Kingdom

Patents ADP number *(if you know it)* 8778631001

If the applicant is a corporate body, give the country/state of its incorporation United Kingdom

4. Title of the invention

Compositions

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Kilburn & Strode
20 Red Lion Street
London
WC1R 4PJ

Patents ADP number *(if you know it)* 125001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

NO

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	8
Claim *(s)*	2
Abstract	0
Drawing *(s)*	0

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature [signature] Date 23/12/03

12. Name and daytime telephone number of person to contact in the United Kingdom

Paul Chapman - Kilburn & Strode
Tel: 020 7539 4200

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.

b) Write your answers in capital letters using black ink or you may type them.

c) If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.

d) If you have answered 'Yes' Patents Form 7/77 will need to be filed.

e) Once you have filled in the form you must remember to sign and date it.

f) For details of the fee and ways to pay please contact the Patent Office.

COMPOSITIONS

The present invention relates to compositions comprising cross linked basic polysaccharides, formed as semi interpenetrating polymer networks and a non cross linked anionic polysaccharide. In particular, the basic polysaccharide is Chitosan or a derivative thereof and the anionic polysaccharide is Hyaluronic acid (HA) or a derivative thereof.

Biocompatible polysaccharide compounds are widely used in the biomedical field. To achieve extended residence times in vivo, these compounds are often chemically modified, usually by crosslinking, to form a polymer network.

One of the most widely used biocompatible polymers for medical use is hyaluronic acid (HA). Being a naturally occurring molecule of the same chemical composition in all vertebrates, it is widely accepted to be virtually free from adverse reactions. Hyaluronic acid is an extremely important component of connective tissue and because of its excellent biocompatibility, it has been the subject of many attempts to crosslink the molecule through both its hydroxyl and carboxyl moieties. However, crosslinking does change the chemical structure of the polymer and , for example, when used in soft tissue augmentation, cells in the connective tissue which are influenced in their development, migration and proliferation by the milieu in which they are found are exposed to a hyaluronic acid polymer network which is not normally found there.

There is increasing evidence in the scientific literature that exogenously administered natural hyaluronic acid stimulates the synthesis of endogenous hyaluronic acid and, therefore, it can be postulated that a biomaterial comprising a biopolymer network whose residence time in vivo could be modified and which at the same time could deliver exogenous hyaluronic acid in its natural non chemically modified structure over an extended period of time would have potential benefits over crosslinked hyaluronic acid in a number of biomedical applicatios. It can be further postulated that

such a biomaterial could have application as a mimetic of the extra cellular matrix if other polysaccharide components of the natural extra cellular matrix such as chondroitin, dermatan and keratin sulphates were incorporated into the polymer network.

Chitosan, an amino group containing basic polysaccharide, a derivative of the biopolymer chitin is well reported in the scientific literature as having excellent biocompatibility and is used in a number of biomedical applications.

US patent No 5,977,330 discloses crosslinked N substituted chitosan derivatives where the substitution is by hydroxyacyl compounds that carry carboxylic acids subsequently crosslinked using polyepoxides. No attempt is made to define a semi IPN using these crosslinked derivatives.

US patent No 6,379,702 discloses a blend of chitosan and a hydrophilic poly(N-vinyl lactam). This document does not disclose any crosslinking of the chitosan or the formation of a semi IPN.

US patent No 6,224,893 discloses compositions for forming semi interpenetrating or interpenetrating polymer networks for drug delivery and tissue engineering whereby the semi IPN is prepared from synthetic and/or natural polymers with a photoinitiator where the crosslinking is initiated by free radical generation by electromagnetic radiation.

US patent No 5,644,049 discloses a biomaterial comprising an interpenetrating polymer network whereby one of the compounds, an acidic polysaccharide, is crosslinked to a second component, a synthetic chemical polymer to create an infinite network. There is no disclosure of crosslinking of acidic polysaccharides with basic polysaccharides.

We have therefore developed a new range of biomaterials, which are based on the formation of a semi IPN with derivatives of cationic polysaccharides which are crosslinked in the presence of anionic polysaccharides under conditions which avoid the formation of ionic complexes between the two polymers and which allow subsequent release of the anionic polysaccharides from the crosslinked network.

Thus, in a first aspect, the present invention provides a composition consisting of a semi interpenetrating polymer network, which comprises at least one crosslinked water soluble derivative of a basic polysaccharide, which has primary and/or secondary amine groups, and a non crosslinked component, which comprises at least one anionic polysaccharide, wherein the anionic polysaccharide resides within the semi interpenetrating polymer network.

A semi interpenetrating polymer network is a combination of at least two polymers formed by crosslinking at least one of the polymers in the presence of but not to the other polymer(s) and having at least one of the polymers in the network as a linear or branched uncrosslinked polymer.

In the context of the present invention, a basic (cationic) polysaccharide is a polysaccharide containing at least one functional group which is capable of undergoing ionisation to form a cation, eg a protonated amine group, while an anionic (acidic) polysaccharide is a polysaccharide containing at least one functional group which is capable of undergoing ionisation to form an anion, eg a carboxylate or sulphate anion.

The compositions of the present invention find use as biomaterials, which can be formulated for instance as hydrogels, which in turn can be placed in soft tissue as a mimetic of the extra cellular matrix.

In one embodiment of this aspect of the invention the water soluble derivative of a

basic polysaccharide is a derivative of chitosan, in particular, N carboxy chitosan, O carboxy methyl chitosan or N, O hydroxyl ethyl chitosan.

In another preferred embodiment, the non crosslinked component is hyaluronic acid. In addition, other anionic polysaccharide components of the extra cellular matrix may be included.

The crosslinked component of the composition can be crosslinked using crosslinking agents such as epoxides, glycidyl ethers or di-isocyanates. In particular, 1,4 butanediol diglycidyl ether (BDDE) can be used. The reaction between the epoxide rings at either end of the BDDE and the amine groups on th echitosan chains occurs by nucleophilic attack by the reactive amine groups with subsequent epoxide ring opening as described in "Chitin in Nature and Technology", R. A. Muzarelli, C. Jeuniaux and G. W. Godday, plenum Press New York 1986, p 303.

The compositions of the present invention can be formed into films, sponges, hydrogels, threads or non woven matrices.

In a second aspect, the present invention provides a method for the preparation of a composition of the invention which comprises crosslinking at least one water soluble derivative of a basic polysaccharide containing primary and/or secondary amine groups, in the presence of at least one anionic polysaccharide, under conditions which avoid protonation of said primary or secondary amine groups and which also avoid reaction of hydroxyl groups or any other functional group on the anionic polysaccharide.

As already discussed, the compositions of the present invention can be formed into various forms of biomaterials for use in medical applications. For instance, to produce an injectible hydrogel:

An aqueous solution of a water soluble derivative of a basic polysaccharide containing primary and/or secondary amine groups is formed, to which is added an anionic polysaccharide. Crosslinking of the basic polysaccharide is then initiated in the presence of a polyfunctional crosslinking agent, under essentially neutral conditions will only crosslink the primary or substituted amines leaving the anionic polysaccharide entrapped within the crosslinked polymer network.

To produce a water insoluble film:

An aqueous solution of a water soluble derivative of a basic polysaccharide containing primary and/or secondary amine groups is formed, , to which is added an anionic polysaccharide. This is allowed to evaporate to dryness. A mixture of a non solvent, such as acetone, and a polyfunctional crosslinking agent is then added and the crosslinking reaction is allowed to take place on the solid phase.

Chitosan becomes soluble in water only when protonated with acids. The polymer thus formed is positively charged and so will interact with negatively charged species such as hyaluronic acid and other polyanions. Such ionic complexes must be avoided in order to form the semi IPN, which is th esubject of the present invention.

Thus, chitosan must be solubilised either as an anionic polyelectrolyte or as a non ionic polymer in either a neutral or a mildly alkaline medium. As already described, suitable derivatives include N carboxymethylchitosan, O carboxy methyl chitosan or N, O hydroxyethyl chitosan. In a preferred embodiment, N carboxy methyl chitosan is used since there is a single primary or secondary amine site for each sugar unit which has a subsequent advantage for accurate characterisation of the biomaterial. Since chitosan and chitin are polymers at the two extreme ends of the continuum of poly(glucosamine) and poly(N-acetyl glucosamine), in another preferred embodiment, the chitosan derivative has a degree of de-acetylation in the range 85% to 95%.

The crosslinking reaction in the presence of the polyfunctional crosslinking agent is generally performed under neutral or mildly alkaline conditions, pH range 7 to 8, which ensures that only the primary or secondary amine groups of the basic polysaccharide can react with the crosslinking agent. Thus, crosslinking of the anionic polysaccharide or indeed crosslinking between the two types of polymer is avoided. The degree of crosslinking can be controlled by varying the molar feed ratio of the basic polysaccharide derivative to crosslinking agent. In this way, the relaease profile of the entrapped anionic polysaccharide can be altered/modified to suit the particular biomedical application in which it is to be used.

Generally, the crosslinking reaction will be carried out at around pH 7.

In a third aspect, the present invention provides a biomaterial comprising a composition of the invention.

In a fourth aspect, the present invention provides the use of a composition or of a biomaterial of the invention in medicine.

In a fifth aspect, the present invention provides the use of a composition of the invention in the preparation of a biomaterial. In particular, the biomaterial is for use in dermatology, plastic surgery, urology and in the field of orthopaedics.

Such biomaterials can be formed into films, sponges, hydrogels, threads or non-woven matrices;

Preferred aspects of each aspect of the invention are as for each other aspect *mutatis mutandis*.

The invention will now be described with reference to the following examples, which should not be construed as in any way limiting.

Example 1

N carboxy methyl chitosan with a degree of deacetylation of 87% (10g) was dissolved in 0.003M NaOH solution. Hyaluronic acid (5g) of approximate molecular weight 2 million g/mol was then added to the above solution. 1,4-butanedioldiglycidyl ether (1g) was added under stirring and the reaction mixture was placed in a water bath at 50°C for two hours. A firm transparent gel was formed which was washed with de-ionised water. The total polymer concentration in the gel was 2.19%.

Immersion of the gel in water after 24 hours showed release of approximately 10% of the hyaluronic acid from the network.

Immersion of the gel in water for a further three weeks resulted in approximately 25% of the starting hyaluronic acid released from the gel network.

Example 2

The polymer blend of example 1 was poured into a petri dish to give a liquid depth of about 6mm. The petri dish was left overnight at 37°C to produce a clear film of N carboxy methyl chitosan and hyaluronic acid.

To this film was added a mixture of 10ml acetone and 1g of 1,4 butanedioldiglycidyl ether and the petri dish was left overnight at 25°C. The resulting opaque crosslinked film was washed with de-ionised water. The film was then cut into pieces approximately 1cm x 1 cm and two pieces were suspended in water each piece being immersed for different times of 6 days and 25 days. The HA concentration of the water was measured and the wet films were weighed and then dried to constant weight to determine the water absorption capacity of the films with the following results:

Immersion period	% HA released	W.A.C %
6 days	10	500
25 days	15	1050

CLAIMS

1. A composition consisting of a semi interpenetrating polymer network, which comprises at least one crosslinked water soluble derivative of a basic polysaccharide, which has primary and/or secondary amine groups, and a non crosslinked component, which comprises at least one anionic polysaccharide, wherein the anionic polysaccharide resides within the semi interpenetrating polymer network.

2. A composition as claimed in claim 1 wherein the water soluble derivative of a basic polysaccharide is a derivative of chitosan.

3. A composition as claimed in claim 2 wherein the derivative of chitosan is N carboxy chitosan, O carboxy methyl chitosan or N, O hydroxyl ethyl chitosan.

4. A composition as claimed in claim 3 wherein the derivative of chitosan has a degree of de-acetylation in the range 85 to 95 %.

5. A composition as claimed in any one of claims 1 to 4 wherein the non crosslinked component is hyaluronic acid.

6. A composition as claimed in any one of claims 1 to 5 wherein the composition also includes one or other anionic polysaccharide components of the extra cellular matrix.

7. A method for the preparation of a composition as defined in any one of claims 1 to 6 which comprises crosslinking at least one water soluble derivative of a basic polysaccharide containing primary and/or secondary amine groups, in the presence of at least one anionic polysaccharide, under conditions which avoid protonation of said primary or secondary amine groups and which also avoid reaction of hydroxyl groups or any other functional group on the anionic polysaccharide.

8. A method as claimed in claim 6 wherein the crosslinking reaction is performed under neutral or mildly alkaline conditions, pH range 7 to 8.

9. A method as claimed in claim 8 wherein the crosslinking reaction is carried out at a pH around 7.

10. A biomaterial comprising a composition as defined in any one of claims 1 to 6.

11. The use of a composition as defined in any one of claims 1 to 6 or a biomaterial as defined in claim 10 in medicine.

12. The use of a composition as defined in any one of claims 1 to 6 in the preparation of a biomaterial.

13. The use as claimed in claim 12 wherein the biomaterial is for use in dermatology, plastic surgery, urology and in the field of orthopaedics.

14. The use as claimed in claim 13 wherein the biomaterial is formed into a film, sponge, hydrogel, thread, or non-woven matrix.